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(54) Abstract Title
Preparation of frozen foods containing antifreeze peptides

(57) A process for the manufacture a frozen food product comprising AFP, wherein a mixture comprising food ingredients and AFP is frozen whereby during or after the freezing process forces are applied to the product by one or more of the following methods:

- (a) application of vibrations to the product,
- (b) application of an hydraulic shock to the product.

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Frozen Food product

Technical Field of the Invention

5 The invention relates to food products containing Anti-freeze peptides (AFPs), in particular to frozen food products containing AFPs.

Background to the Invention

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Anti-freeze peptides (AFPs) have been suggested for improving the freezing tolerance of foodstuffs. In particular it has been suggested that some AFPs may be capable of increasing the smooth texture of frozen food
15 products such as ice cream. Up till now, however the use of AFPs has not been applied to commercially available food products. One reason for this is that up till now it has proved difficult to reproducibly produce a frozen food product having the desired texture and eating
20 characteristics.

Applicants believe that one of the possible reasons for the lack of desired texture in frozen food products containing AFP is that although the AFP is capable of
25 recrystallisation inhibition it is often not capable of avoiding the formation of brittle textures. Applicants believe that one of the explanations for this is that AFPs seem capable of controlling the average particle size of the ice-crystals. However the presence of AFP may also lead
30 to an adverse effect in that the ice-crystals tend to form aggregates leading to hard and brittle products.

Surprisingly it has now been found that if the conditions for producing the frozen food material are carefully chosen, this leads to an improved texture e.g. a reduced hardness and/or brittleness of the product. In particular 5 applicants have found that if specific forces are applied to the product during production this leads to better product properties.

Accordingly in a first aspect the present invention relates 10 to a process for the manufacture a frozen food product comprising AFP, wherein a mixture comprising food ingredients and AFP is frozen whereby during or after the freezing process forces are applied to the product by one or more of the following methods:

- 15 (a) application of vibrations to the product,
 (b) application of an hydraulic shock to the product.

Background to the invention

20 For the purpose of this inventions the term AFP has the meaning such as well-known in the art, see for example "Antifreeze proteins and their potential use in frozen food products", Marilyn Griffith and K. Vanya Ewart, Biotechnology Advances, Vol 13, pp 375-402, 1995.

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Anti-freeze peptides have been described in various literature places. Also these literature places suggest their use in food products, but normally no actual indications are given how to prepare food products of good 30 quality on an industrial scale.

WO 90/13571 discloses antifreeze peptides produced chemically or by recombinant DNA techniques from plants. The AFPs can suitably be used in food-products such as ice cream. Again no guidelines are given as to how to obtain
5 smooth textures.

WO 92/22581 discloses AFPs from plants, which can be used for controlling ice crystal growth. This document also describes a process for extracting a polypeptide
10 composition from intercellular spaces of plants by infiltrating leaves with an extraction medium without rupturing the plant cells.

The present invention aims at providing the food
15 manufacturer a greater flexibility of using AFP material in frozen food products when aiming to obtain a product with improved recrystallisation properties in combination with a good texture. In particular it has been found that the texture of frozen food products containing AFPs can
20 markedly be improved by carefully controlling its production method.

The invention is based of the finding that the application of specific forces to the product during or after the
25 freezing process can lead to less aggregation of ice-crystals in the final product and therewith to an improved texture.

The forces are preferably either repetitive forces applied
30 to the product in the form of vibrations or a sudden concentrated application of force in the form of an hydraulic shock.

EP 584 127 (Acton) describes a method of controlling the solidification of a liquid by subjecting the liquid to ultrasound.

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The vibrational forces can be applied to the product in any way, for example the external product can be subjected to mechanical vibrations, for example by vibrationally shaking it.

10

Alternatively the vibrations can be applied by applying external waves which penetrate the surface of the product. These waves can be longitudinal in nature (e.g. ultrasound) or transverse (e.g. electromagnetic waves such as

15 microwaves, far infrared or extremely high radio waves).

Preferably the frequency of the waves is from 100 kHz to 100 MHz, more preferred from 10 to 100 MHz for longitudinal waves and from 1 to 50 THz, more preferred from 5 to 50 THz for transverse waves. Most preferably those frequencies are

20 chosen which -in the product- lead to wavelengths which are comparable to the average diameter of the ice-crystals which are or have been formed. Advantageously this wavelength is from 0.1 to 100 μm , more preferred from 1 to 50 μm , most preferred from 5 to 25 μm .

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Preferably the power and time of applying the vibrational forces is chosen such that on the one hand aggregation of the ice-crystals is reduced and on the other hand substantial melting of the product is prevented. For

30 example substantial melting of the product may be prevented by using intermittent application of the vibrational forces

for example by using multiple period of vibrations followed by rest-periods.

The vibration can be applied during or after the freezing
5 of the product, preferably during the freezing process e.g. during the hardening stage thereof. In a very preferred embodiment the vibration is applied after pre-freezing e.g. to -4 °C or lower. The time period for applying the vibrations depends on the speed of freezing, the type of
10 vibrations and the moment of applying the vibrations. Generally the total period of the vibrations (excluding the rest-periods) will be from 1 second to 2 hours, more preferred 1 minute to 60 minutes.

15 Alternatively a force may be applied to the product by subjecting the product to a hydraulic shock. Hydraulic shocks can occur in flowing materials when they reach a critical velocity to depth ratio. This shock is equivalent to the sonic boom when travelling at speeds greater than
20 the speed of sound. The occurrence of an hydraulic shock depends on the speed of the flowing material (V), the diameter of the flow tube (d) and the gravitational acceleration (g).

25 For an hydraulic shock to occur:

$$V^2 / (d \times g) > 1$$

Generally it will be sufficient to subject the product to
30 one hydraulic shock during or after the freezing process. If desired the application of the hydraulic shock can be

repeated e.g. from 1 to 1000 times, for example 1 to 10 times.

Frozen food products of the invention may be any food
5 product which can be stored and/or eaten in the frozen
state. Examples of frozen food products which may contain
AFPs are processed food products such as for example frozen
bakery products e.g. doughs, batters, cakes etc., frozen
culinary products for example soups, sauces, pizzas, frozen
10 vegetable products such a compote, mashed potato, tomato
paste etc. A very preferred food product according to the
invention is a frozen confectionery product.

For the purpose of the invention the term frozen
15 confectionery product includes milk containing frozen
confections such as ice-cream, frozen yoghurt, sherbet,
sorbet, ice milk and frozen custard, water-ices, granitas
and frozen fruit purees. Especially preferred products of
the invention are ice-cream and water-ice.

20 Frozen products according to the invention are preferably
aerated. Preferably the level of aeration is more than 50%,
more preferably more than 70%, most preferable more than
90%. Generally the level of aeration will be less than
25 400%, more general less than 300, most preferred less than
200%.

Preferably the level of AFPs in the frozen food product of
the invention is from 0.0001 to 0.5 wt% based on the final
30 product.

The AFP for use in products of the invention can be any AFP suitable for use in food products. Examples of suitable sources of AFP are for example given in the above mentioned article of Griffith and Vanya Ewart.

5

The AFP's can be obtained from their sources by any suitable process, for example the isolation processes as described in the above mentioned documents.

- 10 One possible source of AFP materials is fish. Examples of fish AFP materials are AFGP (for example obtainable from Atlantic cod, Greenland cod and Tomcod), Type I AFP (for example obtainable from Winter flounder, Yellowtail flounder, Shorthorn sculpin and Grubby sculpin), Type II
- 15 AFP (for example obtainable from Sea raven, Smelt and Atlantic herring) and Type III AFP (for example obtainable from Ocean out, Atlantic wolffish, Radiated shanny, Rock gunnel and Laval's eelpout). A preferred example of the latter type is described in WP 97/02343.

20

Another possible source of AFP material are invertebrates. Also AFPs may be obtained from Bacteria.

A third possible source of AFP material are plants.

- 25 Examples of plants containing AFPs are garlic-mustard, blue wood aster, spring oat, winter cress, winter canola, Brussels sprout, carrot, Dutchman's breeches, spurge, daylily, winter barley, Virginia waterleaf, narrow-leaved plantain, plantain, speargrass, Kentucky bluegrass, Eastern
- 30 cottonwood, white oak, winter rye, bittersweet nightshade, potato, chickweed, dandelion, spring and winter wheat, triticale, periwinkle, violet and grass.

Both natural occurring species may be used or species which have been obtained through genetic modification. For example micro-organisms or plants may be genetically modified to express AFPs and the AFPs may then be used in accordance to the present invention.

Genetic manipulation techniques may be used to produce AFPs. Genetic manipulation techniques may be used to produce AFPs having at least 80%, more preferred more than 95%, most preferred 100% homology to the AFP's directly obtained from the natural sources. For the purpose of the invention these AFPs possessing this high level of homology are also embraced within the term "AFPs".

The genetic manipulation techniques may be used as follows: An appropriate host cell or organism would be transformed by a gene construct that contains the desired polypeptide. The nucleotide sequence coding for the polypeptide can be inserted into a suitable expression vector encoding the necessary elements for transcription and translation and in such a manner that they will be expressed under appropriate conditions (e.g. in proper orientation and correct reading frame and with appropriate targeting and expression sequences). The methods required to construct these expression vectors are well known to those skilled in the art.

A number of expression systems may be utilised to express the polypeptide coding sequence. These include, but are not limited to, bacteria, yeast insect cell systems, plant cell

culture systems and plants all transformed with the appropriate expression vectors.

A wide variety of plants and plant cell systems can be transformed with the nucleic acid constructs of the desired polypeptides. Preferred embodiments would include, but are not limited to, maize, tomato, tobacco, carrots, strawberries, rape seed and sugar beet.

10 For the purpose of the invention preferred AFPs are derived from fish or plants. Especially preferred is the use of fish proteins of the type III, most preferred HPLC 12 as described in our case WO 97/02343. From plants especially the use of AFPs from carrot or grass are preferred.

15

For some natural sources the AFPs may consist of a mixture of two or more different AFPs.

Preferably those AFPs are chosen which have significant ice-recrystallisation inhibition properties. This can be measured in accordance to example I.

Preferably AFPs in accordance to the invention provide an ice particle size upon recrystallisation -as measured in accordance to the examples- of less than 20 μm , more preferred from 5 to 15 μm .

Preferably the level of solids in the frozen food product (e.g. sugar, fat, flavouring etc.) is more than 2 wt%, more preferred from 4 to 70wt%.

The method of preparing the frozen food product of the invention can be selected from any suitable method for the preparation of frozen food products. The AFP's can generally be added at various stages of the preparation, 5 for example it can be added in the first pre-mix of ingredients or can later be added during a later stage of the preparation process. For some applications it is sometimes preferred to add the AFP's at a relatively late stage of the production process, for example after 10 (partial) pre-freezing of the product.

The freezing process of the invention will generally include the freezing of the composition say to a temperature of less than -2°C , say from -80 to -5°C . If 15 desired, products of the invention do not need to be subjected to low temperatures to avoid ice-crystal growth. Therefore the products may for example be frozen without the need to use low temperatures say less than -25°C and can also be stored at temperatures which are 20 higher than traditional temperatures to store frozen confectionery products.

Products of the invention are generally characterised by a softer and smoother texture than products which have 25 produced without the application of the forces. This can for example be tested by melting the product and subsequently refreezing it to the same overrun; a softer and smoother texture of the product before melting and refreezing is then characteristic of products of the 30 invention.

For some applications it may be advantageous to include a mixture of two or more different AFPs into the food product. One reason for this can for example be that the plant source for the AFPs to be used, contains more than
5 one AFP and it is more convenient to add these, for example because they are both present in the AFP source to be used. Alternatively it may sometimes be desirable to add more than one AFP from different sources.

10 The invention will now be illustrated by means of the following examples.

Example I

Method of determining whether an AFP possesses ice recrystallisation inhibition properties.

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Recrystallisation inhibition properties can be measured using a modified "splat assay" (Knight et al, 1988). 2.5 μ l of the solution under investigation in 30% (w/w) sucrose is transferred onto a clean, appropriately labelled, 16 mm circular coverslip. A second coverslip is placed on top of the drop of solution and the sandwich pressed together between finger and thumb. The sandwich is dropped into a bath of hexane held at -80°C in a box of dry ice. When all sandwiches have been prepared, sandwiches are transferred from the -80°C hexane bath to the viewing chamber containing hexane held at -6°C using forceps pre-cooled in the dry ice. Upon transfer to -6°C , sandwiches can be seen to change from a transparent to an opaque appearance. Images are recorded by video camera and grabbed into an image analysis system (LUCIA, Nikon) using a 20x objective. Images of each splat are recorded at time = 0 and again after 60 minutes. The size of the ice-crystals in both assays is compared. If the size at 30-60 minutes is similar or only moderately (less than 10%) increased compared to the size at $t=0$, and/or the crystal size is less than 20 micrometer, preferable from 5 to 15 micrometer this is an indication of good ice-crystal recrystallisation properties.

Example II

Ice cream of the following formulation was produced in a conventional ice-cream freezer (W104 supplied by APV). The ice-cream exited the freezer at a temperature of -5 C having an overrun of 100% and was subsequently hardened to -25 C in a blast freezer for 4 hours. Ultrasound of frequency 200 kHz was applied during the hardening process, whereby the intermittent application periods of 5 seconds were used followed by a 25 second rest-period.

Ingredient	parts by weight
Butteroil	10
Sugar	15
Skimmed milk powder	10
Locust bean gum	0.2
monoglycerides	0.2
AFP*	0.01
Water	balance

As a comparison the same ice-cream was produced, except no ultrasound was applied during hardening.

15

After storage for 24 hours at -18 C the ice-cream according to the invention was markedly softer and smoother than the comparative ice-cream

20 Note AFP is AFP HPLC-12 as described in WO 97/02343

Example III

Example II was repeated by applying instead of ultrasound electro-magnetic waves of a frequency of 30 Thz during the hardening process. Again the waves were applied during intermittent periods of 5 seconds followed by 25 seconds of rest.

Again a product was obtained which was softer and smoother than the comparative product.

Example IV

The ice-cream of example II was produced at a rate of 360 litres/hour (mix flow i.e. excluding overrun). On exiting the freezer the mix at -5 C was pumped through pipework of 2.5 cm internal diameter, followed by a converging-diverging nozzle. This nozzle had a smallest internal diameter of 1.3 cm.

20

The ice-cream experienced a hydraulic shock within the converging-diverging nozzle. The exiting ice-cream was then hardened in a conventional hardening tunnel and stored at -25 C.

25

As a comparison the same ice-cream was produced except that this was not pumped through the nozzle. The product according to the invention was markedly softer and smoother than the comparative product.

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Claims

1. A process for the manufacture a frozen food product comprising AFP, wherein a mixture comprising food ingredients and AFP is frozen whereby during or after the freezing process forces are applied to the product by one or more of of the following methods:
 - (a) application of vibrations to the product;
 - (b) application of an hydraulic shock to the product.
2. A process according to claim 1, wherein the vibration involves the application of external waves which penetrate the surface of the product.
3. A process according to claim 2, wherein the waves are longitudinal waves.
4. A process according to claim 3, wherein the waves have a frequency of from 100 kHz to 100 MHz.
5. A process according to claim 2, wherein the waves are transverse.
6. A process according to claim 5, wherein the waves have a frequency of 1 to 50 THz.
7. A process according to claim 1, wherein the food product is a frozen confectionery product.
8. A process according to claim 1, wherein the level of AFP is from 0.001 to 0.5 wt%.